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Applicant:	Arthur M. Krieg et al.
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CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to MAIL STOP AF, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the 28 day of November, 2005.

Helen C. Lockhart

MAIL STOP AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION OF DR. GEORGE T. DE SANCTIS

I, Dr. George T. De Sanctis declare as follows:

1. I am Director of Respiratory Pharmacology at sanofi-aventis, a licensee of the above-identified patent application and I make this declaration in support of that application. I have extensive knowledge and experience in the area of Respiratory disease. In support of this, I have attached a copy of my resume for review.

2. I have reviewed the above-identified patent application ("Krieg application") and the pending claims. I believe that one of ordinary skill in the art reviewing the data in the patent application would have expected that virtually all oligonucleotides having

an unmethylated CpG motif would have the ability to induce cytokine production that would drive the immune system towards a Th1 response.

3. At least 40 oligonucleotides were tested in the Krieg application for activity *in vitro* using mouse cells. The data, which is shown in Tables 1-3, establishes that unmethylated CpG is responsible for the cytokine induction. The data presented in Table 4 confirmed that the activity observed with CpG oligonucleotides *in vitro* translated into immune activation *in vivo* in mice.

4. It was well known in 1996 that the immune system of a mouse was a good model for the immune system of a human. The Krieg application, however, conducted experiments on human cells to confirm that the CpG effects in mice would also be observed in humans. Eleven different CpG oligonucleotides were tested for the ability to induce cytokine secretion (5 different cytokines) by human cells *in vitro*. The data, which is shown in Table 5, was consistent with the mouse studies in that multiple CpG oligonucleotides were capable of influencing the immune response to produce a cytokine profile consistent with driving the immune system toward a Th1 response.

5. The data from Tables 1-5 are sufficient to support a conclusion by one of skill in the art that CpG oligonucleotides would have the ability to induce cytokine production that would drive the immune system towards a Th1 response.

6. According to the Krieg application, the experiment to which Table 13 relates involved the induction of IL-12 by human PBMC using a panel of CpG oligonucleotides. The data shown in Table 13 was a selection of only two subjects from the experiment, selected to demonstrate the extremes of the possible variability between subjects (specification page 41 lines 5-8). The variability observed in the data would have been expected and was consistent with the variability of responses produced by many drugs in humans.

7. Humans are an outbred population, unlike mice used in experimental research (bred to be identical). It was known in 1996 that humans have an immune status that fluctuates much more than that of mice used in experimental research (which are kept in carefully controlled environments and used at a particular age). It was known in 1996 that human's age, health, medications, status in fighting exposure to an infectious agent, genome and even psychological state determine the immune status and the way and extent to which humans will respond to a drug. The immune status of the individuals selected in Table 13 were not reported, and that status alone could have accounted for the variability.

8. Additionally, it was known in 1996 that humans produce relatively low levels of cytokines like IL-12. It was known in 1996 that IL-12 is part of complex interdependent pathways, including autocrine stimulatory pathways in which the IL-12 could act on its receptor without being detectable in a culture supernatant. The absence of detectable levels of IL-12 in the patient identified as experiment 2 in Table 13 does not mean that Th2 to Th1 switching was not initiated or that IL-12 was not at all involved. One of ordinary skill in the art in 1996 would have understood the selection of two subjects at the extremes to confirm only the predictable variability in the immune response among different individuals.

9. Further, I believe that one of ordinary skill in the art in 1996 reviewing the description and data in the Krieg application would have expected that oligonucleotides having an unmethylated CpG motif would have the ability to induce a therapeutic response in an asthmatic subject, whether the oligonucleotide was administered with or without an antigen.

10. The data, described above and presented in the patent application, examining shifts in cytokine induction, were achieved using CpG alone without an allergen. For instance and as discussed above, CpG oligonucleotides were used alone without antigen/allergen to produce Th1 biased cytokine induction in Table 5. No antigen

was administered. Th1 cytokines include IFN-γ, TNF-α, IL-12, and GM-CSF. One of ordinary skill in the art would have expected, based on these data, that the administration of CpG oligonucleotides alone to an asthmatic subject would help restore a proper cytokine balance that would be therapeutically useful.

11. Asthma is a condition that currently is treated only after it has been diagnosed. The symptoms typically will arise when a person is exposed to an agent or to conditions that initiate the aberrant response. Administration of CpG oligonucleotide (as a mono-therapy) would have been expected to act on the immune system to bias the cytokine profile away from a Th2 response, keeping the immune system 'ready' for the moment when it next encounters the agent or conditions.

12. The data presented in Example 12 of the Krieg application further confirmed that a CpG containing oligonucleotide not only would shift the pattern of cytokine release *in vivo*, but would also treat asthma. An important aspect of Example 12 was establishing that the expected *in vivo* shift of the pattern of cytokines toward Th1 would translate into a therapeutic benefit. Based on the data in the specification, including the data of Example 12, one of ordinary skill in the art would have believed that CpG not only shifts cytokine response, but would be effective in influencing important therapeutic aspects of asthma, such as infiltration of cells and fluid into the lungs.

13. The data of Example 12 are consistent with the other data and teachings found in the specification. The data further demonstrate that CpG oligonucleotides produce a therapeutic effect, e.g. reduction in eosinophil influx, even in the circumstance that the CpG oligonucleotide is administered at the same time as the Schistosoma mansoni eggs. The eggs (or antigen) when administered alone would have resulted in the production of a TH2 immune response associated with the production of IgE. Thus, the data demonstrate that the CpG oligonucleotides were able to drive a

therapeutically favorable response even when delivered at the same time as the eggs that otherwise would have caused an allergic response.

14. Asthma, including allergic asthma, involves both the "innate" (not antigen specific) and the "adaptive" (antigen specific) arms of the immune system. The data in the Krieg application demonstrated *in vitro* and *in vivo* that the innate immune system could be biased toward Th1 in the absence of antigen. The Krieg application went further, however, demonstrating not only that innate arm could be biased toward Th1, but also that this could occur even in the presence of a Th2 stimulatory agent

15. We have tested at sanofi-aventis the therapeutic effect of a CpG oligonucleotide (5'TCG TCG TTT TGA CGT TTT GTC GTT3') having a phosphorothioate backbone in a nonhuman primate asthma model, against established airway hyperresponsiveness. This model was known in 1996 and the CpG oligonucleotide tested was one selected as described in the Krieg application.

16. Wild caught cynomolgus monkeys (*Macaca fascicularis*) are naturally allergic to *ascaris suum* antigen, and upon inhaled challenge with this antigen exhibit an IgE-mediated response (3, 4). This response is characterized by an acute bronchoconstriction, with the development of airway hyperresponsiveness, a hallmark of human asthma (5). As with human asthma, the acute response can be reversed by beta-agonists and the airway hyperresponsiveness and inflammation can be ameliorated by systemic steroids (6, 7). Recently marketed newer therapies for asthma (e.g., the leukotriene antagonist montelukast ('Singulair') and those currently in clinical trials (e.g., Phosphodiesterase IV Inhibitors) have also shown efficacy in nonhuman primate asthma models (8-10). Furthermore, primates, like humans, are thought to have a TLR9 distribution restricted to B lymphocytes and plasmacytoid dendritic cells. All these features make a nonhuman primate asthma model relevant to human asthma.

17. The study was carried out according to a nonhuman primate chronic allergen challenge model Protocol. A schematic of the Protocol is shown in Figure 1. This type of Protocol was known in 1996 A vehicle control group and 2 CpG oligonucleotide treatment groups were established – a high dose group (300ug, total lung dose) and a low dose (75ug, total lung dose) group. Based on the lung exposure from a 4 week Drug Safety and Evaluation study in primates, this lower dose was estimated as equivalent to an achievable inhaled dose in man of 6mg after adjustment for the differences in the efficiency of the delivery devices and the comparable lung weights between species.

18. Airway Hyperresponsiveness (AHR) Methods: Airway hyperresponsiveness was measured by performing a dose response curve with inhaled methacholine (range 0.1 to 100mg/ml) and determining the PC_{100} – the dose of methacholine which caused a 100% increase in baseline lung resistance. Before exposure to antigen, animals will have a relatively high PC_{100} (usually between 10 and 100mg/ml). After antigen exposure when AHR has developed, a much lower dose of methacholine is required to achieve the PC_{100}. Because of individual animal variation in PC_{100} values, the results are often normalized with respect to the baseline (pre-antigen exposure) value (100%), with subsequent PC_{100} measurements being reported as a percentage of that value. The aim of a therapeutic treatment is to reverse the antigen-induced AHR such that the PC_{100} returns to a point near to it's starting value of 100%. Achieving a PC_{100} of greater than 100% after therapy would indicate that the animal is even less hyperresponsive than at the start of the study – a substantial clinical benefit.

19. Briefly, baseline airway hyperresponsiveness (AHR) was measured on Day 0. AHR was measured again on Day 10, by which time the animals had received 3 antigen challenges, enough to make them hyperresponsive.

20. Treatments began on day 11, both vehicle and drug being administered by aerosol. Treatments were administered once weekly for 4 weeks, with antigen challenge

being maintained throughout. AHR was assessed on Day 24 (after 2 treatments) and again on Day 38, at the conclusion of the study (and after 4 treatments).

21. Airway hyperresponsiveness Results: The data are shown in Figure 2. AHR was measured on Days 0, 10, 24 and 38. The PC_{100} values on Days 10, 24 and 38 were expressed as a percentage of the Day 0 value. At Day 10, all the groups are hyperresponsive, as shown by their reduced PC_{100}. Following treatment, there is a dose-related reversal of airway hyperresponsiveness so that by day 38 (after 4 treatments) there is a significant increase in PC_{100} compared to Day 10, in both treatment groups. However, the control group remains hyperresponsive, as the Day 38 PC_{100} is not significantly improved compared to Day 10.

22. It can be noted from the size of the error bars (standard deviation) that there is a degree of heterogeneity in the response. However, 13/16 (81%) animals in the treated groups responded to CpG, with the remaining 3 animals apparent 'non-responders'. Polymorphisms in TLR9 have been identified in humans (12) and similar polymorphisms likely exist in cynomolgus monkeys, which may contribute to this heterogeneity. This degree of variability in response to therapeutics is not uncommon in primate studies (nor in humans) and has previously been documented for studies with leukotriene receptor antagonists (13), anti-histamines (7) and phosphodiesterase 4 inhibitors (9); in each of these studies, examination of the individual animal data for AHR shows at least 1 non-responder in the treated groups.

23. Nonetheless, the majority of the animals respond to therapy in a statistically significant manner, and the degree of variability in the above primate study with the CpG oligonucleotide is similar to the other studies cited.

24. There is a high degree of similarity between the responses to therapeutics in the primate asthma model, and the responses of patients. With steroid treatment (e.g. beclomethasone, dexamethasone, budesonide) there was little or no effect on the

acute bronchoconstriction, while AHR was inhibited 58-100% in both humans and primates. Steroids also reduced the late airway response in humans (63-81%) and primates (60%) and reduced bronchoalveolar lavage eosinophils in the primate (44-100%) and sputum eosinophils (64%) in humans (7, 14, 15, 16, 17). Preclinical trials with leukotriene antagonists including montelukast ('Singulair') in the primate asthma model showed that it could inhibit the acute bronchoconstriction by about 70%, the late airway response by 60% and the airway hyperresponsiveness by 80-90%. The corresponding data from clinical trials was 56% inhibition of the acute bronchoconstriction, 60% for the late airway response and 50-100% for the AHR (8, 16, 18), thus showing the good predictiveness of the primate asthma model.

25. The incidence and severity of asthma is steadily increasing in the developed but not in the developing countries. This increase in the developed countries is believed to be due in part to the steady decline of infectious diseases. The rationale behind this theory, known as the "Hygiene Hypothesis", is that exposure to infectious organisms or exposure to antigens derived from these pathogenic organisms induce a T-helper 1, or Th1 response, early in life shifting the immune response of individuals with an allergic predisposition away from a Th2 response towards a Th1 response (IL-12, INF-g), thereby conferring protection from developing asthma. This theory was known in 1996. It has since been tested in animal studies where, for example, infection of the lung with live Mycobacterium bovis bacillus Calmette-Guerin (BCG) results in suppression of key asthma phenotypes in mice, specifically, induction of airway hyperresponsiveness and airway eosinophilia (1,2).

26. It was recognized in the Krieg application that unmethylated CpG motifs found in the bacterial DNA invoke Th1 responses. The Krieg application taught that this immune response can also be mimicked by administration of synthetic oligonucleotides containing unmethylated CpG motifs. These synthetic oligonucleotides containing unmethylated CpG motifs would have been expected,

based on the Krieg application, to function as effective immune modulators in-vivo, the goal of which is to suppress the generation of asthma-promoting Th2 responses by several mechanisms, such as establishing a long-lasting Th1 memory response. These immunostimulatory synthetic oligonucleotides would have been understood to mimic what occurs naturally in nature, via infectious agents.

27. I declare that all statements herein of my own knowledge are true and that all statements made on information and belief are believed to be true. I declare further that the statements were made with the knowledge that willful, false statements and the likes so made, are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of this document and any patent which may issue from the above-identified patent application.

Date: NOV 28th, 05

George T. De Sanctis, Ph.D., F.C.C.P.

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Figure 1

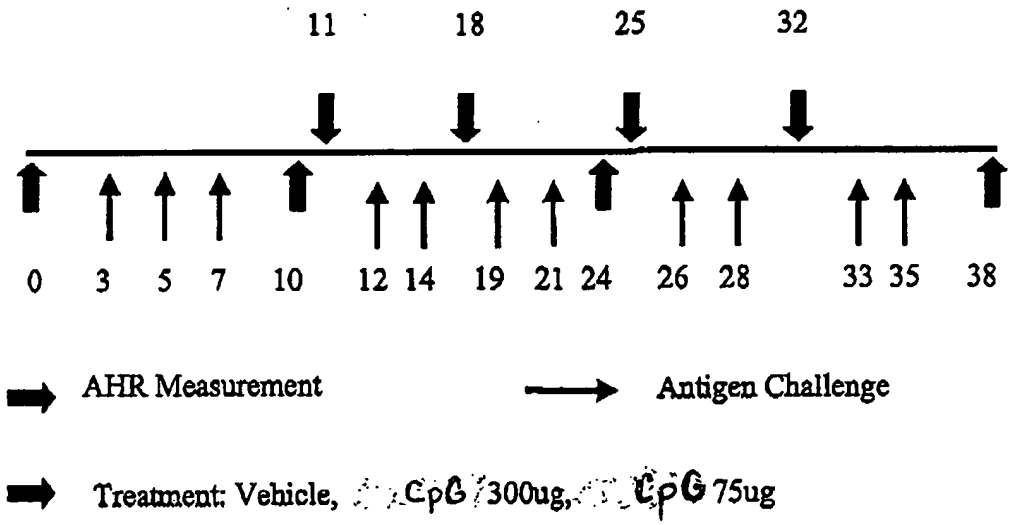
11
18
25
32
0
3
5
7
10
12
14
19
21
24
26
28
33
35
38
AHR Measurement
Antigen Challenge
Treatment: Vehicle, CpG 300ug, CpG 75ug

Figure 2

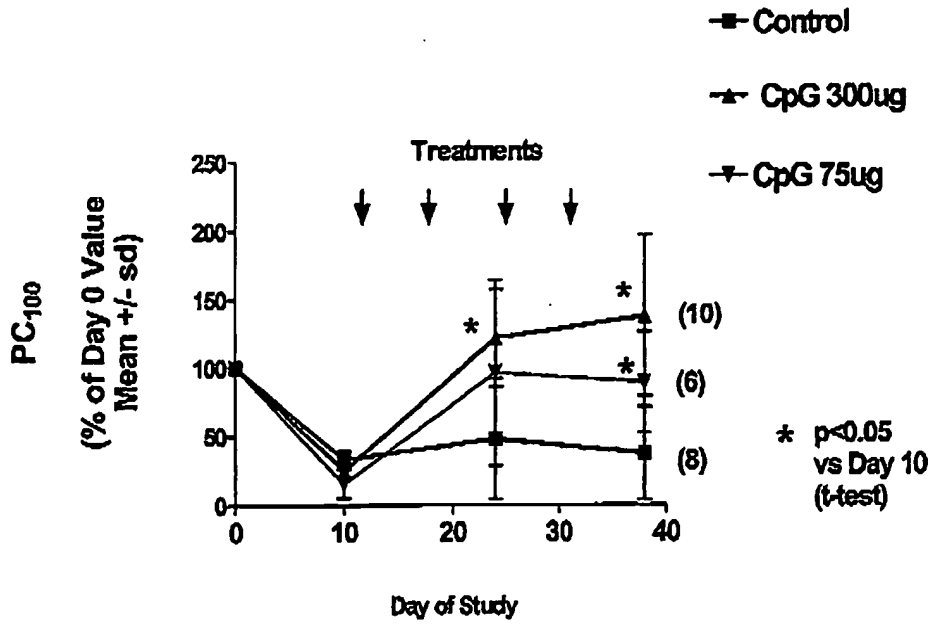
Control
CpG 300ug
CpG 75ug
Treatments
PC100
(% of Day 0 Value
Mean +/- sd)
250
200
150
100
50
0
0
10
20
30
40
Day of Study
*
*
*
(10)
(6)
(8)
* p<0.05
vs Day 10
(t-test)

EXHIBIT 2

November 22, 2005

CURRICULUM VITAE

Name:	George Tilo De Sanctis, Ph.D., F.C.C.P.
Current Position:	Director, Respiratory Pharmacology/Pathology Sanofi-Aventis Pharmaceuticals, Inc.
Office Address:	8 Titus Road Skillman, NJ 08558
Place of Birth:	Guatemala City, Guatemala
Citizenship:	Canadian (Permanent Resident in US)
Languages Spoken:	English, French, Italian
Children:	Diana "Tucker" (6), Camden (4), Devon (9 months)
Home Telephone:	609-333-8520
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Education:

1983	B.Sc.	McGill University
1986	M.Sc.	McGill University
1991	Ph.D.	University of Calgary

POSTDOCTORAL TRAINING:

1991-1992	Research Fellow, Pulmonary Medicine, University of Alberta
1992-1995	Research Fellow, Pulmonary Medicine, Brigham and Women's Hospital
1992-1995	Research Fellow, Pulmonary Medicine, Harvard Medical School

ACADEMIC APPOINTMENTS:

1991-1995	Research Fellow, Pulmonary Medicine, Harvard Medical School -
1996	Research Associate in Medicine, Harvard Medical School
1996-1998	Instructor in Medicine, Harvard Medical School
1998-2001	Assistant Professor of Medicine, Harvard Medical School

HOSPITAL APPOINTMENTS:

1992-1995	Research Fellow in Medicine, Brigham and Women's Hospital
1995-2001	Research Associate in Medicine, Brigham and Women's Hospital

PROFESSIONAL SOCIETY INVOLVEMENT:

1990-	American Thoracic Society	Member

EDITORIAL BOARDS:

1996-	ad hoc reviewer	Nature Genetics
1997-	ad hoc reviewer	Clinical and Experimental Allergy
1999-	ad hoc reviewer	European Respiratory Journal
1999-	ad hoc reviewer	American Journal of Respiratory Cell and Molecular Biology
1999-	ad hoc reviewer	The Lancet
1999-	ad hoc reviewer	Nature Medicine
1999-	ad hoc reviewer	Journal of Clinical Investigation
1999-	ad hoc reviewer	Journal of Allergy and Clinical Investigation

AWARDS AND HONORS:

1979	Asbestos Corporation Scholarship, Bishop University
1983-86	Canadian Tobacco Manufacturing Council (C.T.M.C.) MSc Studentship , McGill University
1988	University of Calgary Faculty of Medicine Trust Fund Award
1989-91	Alberta Lung Association Doctoral Studentship Award, University of Calgary,

1991	Best Basic Research Paper Award, Annual Pathology Residents and Graduate Students Research Day, University of Calgary
1991-92	Glaxo/Medical Research Council of Canada Postdoctoral Fellowship Award
1991-93	Canadian Lung Association Postdoctoral Fellowship Award
1992	Dupont/ American College of Chest Physicians Young Investigator Award
1992-95	Medical Research Council of Canada Postdoctoral Fellowship Award
1996	Edward Livingston Trudeau Scholar Award, American Lung Association (Highest Ranking Research Grant Awards)
1996	American College of Chest Physicians Young Investigator Award
1997	Elected Fellow of the American College of Chest Physicians (ACCP)
1998	"Partners Investigator Nesson Award", Brigham & Women's Hospital and Massachusetts General Hospital
1999	"Partners Investigator Nesson Award", Brigham & Women's Hospital and Massachusetts General Hospital

INDUSTRIAL AFFILIATIONS:

1. Consultant: Boehringer Ingelheim Pharmaceuticals, 1997-1998
2. Consultant: ICAgen Pharmaceuticals, 2000-2001
3. Elected Member, Board of Directors: Medical Area Federal Credit Union, 1999- 2001

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INVITED REVIEWS AND BOOK CHAPTERS

1. Invited Review Article
   Tomkiewicz RP, Albers GM, **De Sanctis GT**, Remirez O, King M, and Rubin BK. Species Differences in the Physical and Transport Properties of Airway Secretions. Can J Physiol Pharmacol 1995, (73):165-171

2. Invited Review Article
   Tomkiewicz RP and **De Sanctis GT**. New approaches in cystic fibrosis. In: Advance for Managers of Respiratory Care 1996, 5(6): 21-24

3. Invited Book Chapter
   Davidovitch Z, Godwin SL, Park Y, Taverne AR, Dobeck JM, Lilly CM, and **De Sanctis GT**. The etiology of root resorption. In: McNamara JA, ed. Orthodontic Treatment: The Management of Unfavourable Sequelae. Ann Arbor: U Michigan Press, 1996: 93-117

4. Invited Book Chapter
Dasgupta B, Tomkiewicz RP, **De Sanctis GT**, Boyd WA and King M. Rheological properties in cystic fibrosis airway secretions with combined rhDNASE and gelsolin treatment. In: Singh M and Saxena VP, ed. Advances in Physiological Fluid Dynamics. New Delhi: Narosa Publishing House, 1996 :74-78

5. Invited Book Chapter
Drazen JM, Finn PW and **De Sanctis GT**. Mouse Models of Airway Responsiveness: Physiological Basis of Observed Outcomes and Analysis of Selected Examples Using These Outcome Indicators. Annual Review of Physiology 1999, 13(61):593-625

6. Invited Review Article
De Sanctis GT and Drazen JM. Genetics of airway responsiveness in the inbred mouse. Research in Immunology 1997, 148(1):73-79

7. Invited Review Article
De Sanctis GT and Drazen JM. Genetics of native airway responsiveness in mice. Am. J. Resp. Crit. Care Med. 1997, 156:1-7

8. Medical Review Journal
De Sanctis GT, Itoh A, Green FHY, Qin S, Kimura T, Grobholz JK, Martin TR, Maki T, and Drazen JM. T-lymphocytes regulate genetically determined airway hyperresponsiveness in mice. Allergy Up To Date 1997, 4(6): 10-12
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9. Invited Review Article
In KH, Silverman ES, Asano K, Beier D, Fischer AR, Keith TP, Serino K, **De Sanctis GT**, and Drazen JM. Mutations in the human 5-lipoxygenase gene. Clinical Reviews in Allergy and Immunology 1999, 17: 59-69

10. Invited Review Article
Drazen JM, Takebayashi T, Long NC, **De Sanctis GT**, and Shore SA. Animal Models of Asthma and Chronic Bronchitis. Clin Exp Allergy 1999 Jun;29(S2):37-47

11. Invited Review Article
Yandava CN and **De Sanctis GT**. The Genetics of Asthma. Clinical Pulmonary Medicine 2001, 8:59-65

12. Invited Review Article
De Sanctis GT, Daheshia M and Daser A. Genetics of airway hyperresponsiveness. J Allergy Clin Immunol. 2001, 108 (1), 11-20

13. Invited Review Article
Daser A, Daheshia M and **De Sanctis.GT**. Genetics of allergen-induced asthma. J Allergy Clin Immunol 2001, 108(2), 167-174

14. Invited Book Chapter
Deykin A, Massaro AF, Kobzik L, **De Sanctis GT**, and Drazen JM. Molecular and Cellular Sources of Exhaled Nitric Oxide. In: Marczin, ed. Disease Markers in Exhaled Nitric Oxide. Lung Biology in Health and Disease: Exec Ed. Claude Lenfant, Marcel Dekker, Inc., 2002;73-90

15. Invited Book Chapter
De Sanctis GT and Haddad El-B. Contribution of Nitric Oxide Synthase Isoforms to Allergen-Induced Airway Hyperresponsiveness and Inflammation: Lessons from Murine Models. In: Eissa NT and Huston DP, ed. Therapeutic Targets in Airway Inflammation. Lung Biology in Health and Disease: Exec Ed. Claude Lenfant, Marcel Dekker, Inc., 2003:143-159

1. Letters
De Sanctis GT. Canada-bashing [letter] Can Med Assoc J 1993; 148(12): 2110

Selected External Presentations

1. Invited Speaker: McGill University Centre for the study of Host Resistance June 1995, "Genetics of Airway Hyperresponsiveness in the mouse"

2. Invited Speaker: "Johns Hopkins Asthma Center" April 1995, "Genetics of Airway Hyperresponsivess"

3. Invited Speaker: Asthma 95: Theory to Treatment, "Genetics of Airway Responsiveness in C57BL/6 and A/J mice: A Segregation and Quantitative Trait Locus Analysis".

4. Invited Speaker: Twelfth Transatlantic Airway Conference January 1997 "Genetics of Native Airway Responsiveness in Mice"

5. Invited Speaker: American Academy of Allergy, Asthma and Immunology March, 2000 , NIAID Symposium"Genetics of Airway Hyperresponsiveness".